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Immunosuppressive Activity of BCG: Effects of Adjuvant Disease, Lymphocyte Subpopulations, and Homing of Thoracic Duct Cells in Rats

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Administration of BCG by various dosage schedules suppressed adjuvant disease in rats. BCG administration produced an initial increase, followed by a depression, of the phytohemagglutinin response of purified blood lymphocytes. An increase in absolute and relative numbers of bursa-equivalent (B)-cells followed BCG administration, concurrent with a decrease in the phytohemagglutinin responsiveness. With adjuvant alone, there was a diminution in phytohemagglutinin response and an increase in number of B-cells; the latter occurred immediately after adjuvant injection and also when the generalized disease appeared. When both BCG and adjvant were present, parallel increases of phytohemagglutinin responsiveness and B-cell numbers resulted. The pattern of tissue localization of radioactively labeled thoracic duct cells from normal or BCG-treated donors given to normal, BCG-treated, adjuvant-injected, and BCG-treated + adjuvant-injected syngeneic recipients indicated significantly greater homing to the thymus and decreased localization to the bone marrow when BCG had been given to either donors or recipients. When labeled thymus cells were used, only the decreased bone marrow localization was noted. These observations suggest that the suppressive effect of BCG may be mediated through modification of the lymphocyte recirculation pattern, possibly resulting from alterations in lymphocyte recognition sites.

We investigated the effect of BCG on experimentally induced adjuvant disease (AD), a noninfectious inflammatory polysystemic syndrome produced in rats (30, 44), and noted that it suppresses the disease. AD has been reported to be suppressed by a variety of mechanisms (9-11, 14, 15, 19, 30, 31, 36-38, 44, 46, 49). Some of the immunologically specific factors thought to be involved in the suppression of the disease are antigenic competition (10), neonatal tolerance (9, 46), adult tolerance (14) or immunological paralysis (31), and alterations of the host response. AD is also nonspecifically affected by anti-inflammatory and immunosuppressive agents (11, 31, 36, 37, 49) and by an interferon-mediated effect (15). BCG is known to affect cellular processes involved in both afferent and efferent components of the immune response (1, 3, 22, 24, 27, 41, 47). BCG has been shown in mice to activate suppressor cells in bone marrow (5), and also to produce a depression of the response to various mitogens in culture (43). To elucidate the mechanism by which BCG produced disease suppression in this nonmalignant disease model, several experiments were carried out. To show that the effect of BCG is independent of the presence of mycobacterial components in the adjuvant, we investigated its suppressive capacity on AD when the adjuvant mixture contained corynebacteria instead of mycobacteria. To study the interactions in rats between BCG and the cellular immune system, purified protein derivative (PPD) reactivity, and lymphocyte subclasses, some of the functions, distribution, and in vitro mitogen responsiveness of lymphocyte subclasses (with and without BCG treatment) were determined.

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MATERIALS AND METHODS

AD was induced in inbred adult male Wistar-Furth (WF) or Fisher 344 rats, 180 to 250 g (Microbiological Associates, Bethesda, Md.), by intradermal injection of 250 μg of heat-killed *Mycobacterium tuberculosis* (strains PN, DT, and C) in 0.05 ml of sterile mineral

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oil (always given at day 0), and the arthritis was scored on a four-point scale (0 to 3) as described (36). Experimental groups consisted of 5 to 20 rats (see Fig. 1 and 2).

BCG (BCG-s Frais, Pasteur Institute, Paris, France, from where it was obtained at biweekly intervals), 25 mg suspended in 0.5 ml of sterile saline, was given intraperitoneally to each rat according to one of three administration schedules, as follows: (i) pretreatment—BCG on days −6, −3, and 0; (ii) continuous administration—BCG as for pretreatment plus twice-weekly injections for 7 weeks; (iii) postadjuvant injection treatment—BCG three times at 3-day intervals beginning on day +5 (prearthritis phase), +12 (rapidly increasing disease), or +19 (disease plateau).

Hemoglobin values and leukocyte and differential counts were determined by standard methods on tail vessel blood (see Fig. 3 and 4). Blood lymphocytes were isolated by centrifugation on a Ficoll-Hypaque mixture (Pharmacia Fine Chemicals, Uppsala, Sweden, and Winthrop Laboratories, New York, N.Y., respectively) at times indicated (see Fig. 4 and 5). Complement receptor-bearing lymphocytes (CRL) (the major B-cell compartment in the rat) were quantitated in this lymphocyte-enriched preparation by the formation of rosettes with sheep erythrocytes coated with amboceptor and complement (EAC) (7) with the modifications described (33). Stimulation studies were done on triplicate sets of lymphocytes cultured with phytohemagglutinin (PHA, reagent grade, Wellcome Laboratories, Beckenham, England) in concentrations of 100, 10, or 1 μg/ml of cell suspension (see Fig. 7), as described (33, 42).

The distribution of thoracic duct (TD) lymphocytes (or of thymocytes) collected from normal (groups 1 through 4) and BCG-treated (groups 5 through 8) donor rats was determined in normal (groups 1 and 5), BCG-treated (groups 2 and 6), adjuvant-injected (groups 3 and 7), and BCG-treated plus adjuvant-injected (groups 4 and 8) syngeneic recipient rats, three per group (see Table 1). TD drainage was performed as described (35). The cells were labeled (17) with $Na^{51}CrO_4$ (E. R. Squibb & Sons, Ltd., New Brunswick, N.J.) and washed in RPMI-1640 as described (34). TD lymphocytes (2.5×10^7) in 0.5 ml of RPMI-1640 were injected intravenously into each rat. After 5 days the rats were killed by exsanguination, and the specimens listed in Tables 1 and 2 were removed and counted in a Nuclear-Chicago 2-channel automatic gamma counter series 1185 (Nuclear-Chicago, Des Plaines, Ill.).

Delayed hypersensitivity as reflected by inflammatory response to intradermal injections of 5 μg of PPD in 0.3 ml of diluent (Merck, Sharp and Dohme, West Point, Pa.) was determined on day +14 in BCG-treated, adjuvant-injected, BCG-treated plus adjuvant-injected, and control rats as described (35, 49) (see Table 1).

Because of the observation that disease progression occurred in the treated groups after BCG therapy was stopped, we studied the effect of prolonged BCG treatment (two injections per week for 2 weeks) on the course of AD. WF rats were injected with adjuvant on day 0, then divided into three groups of five each. Group 1 received saline. Groups 2 and 3 received intraperitoneal injections of BCG twice weekly beginning on days 6 and 12, respectively, for 2 weeks. Their arthritis scores and weights were recorded daily.

Since tolerance (or paralysis) or cross-reactivity between BCG and *M. tuberculosis* may influence the course of AD, arthritis was also induced using *Corynebacterium rubrum* instead of *M. tuberculosis* in the adjuvant mixture. Fifteen adult WF rats were given, in the left hind paw on day 0, intradermal injections of an adjuvant mixture containing 600 μg of *C. rubrum* in 0.05 ml of sterile mineral oil (29).

RESULTS

Effect of BCG on the development of joint manifestations. (i) Effect of BCG pretreatment on arthritis. It can be seen in Fig. 1 that nontreated, adjuvant-injected controls developed arthritis by day 10, with maximal involvement between days 13 and 25. BCG pretreatment (Fig. 1) strikingly reduced the arthritis score from the maximum of 5.0 per rat in the adjuvant-injected group to less than 1.0 per rat ($P < 0.01$ on day 22), and delayed onset of the disease. No late flare of arthritis was observed in this group by day 100.

BCG injections given twice weekly over the initial 50 days after adjuvant completely prevented arthritis to day 88 (Fig. 1), when 3 of 10 rats developed mild disease which lasted for a few days only.

(ii) Effect of short-term BCG treatment after disease induction. When BCG was given on days 5, 8, and 11 (just before arthritis was evident in the control group) (Fig. 2), delayed onset and significant suppression of the initial polyarthritis ($P < 0.02$ on day 14) were noted. BCG given on days 12, 15, and 18 during the most active phase (Fig. 2) improved the arthritis within 4 h of the injection, and the improvement lasted for the period of BCG administration ($P < 0.02$ on day 19). BCG given later, on days 19, 22, and 25, during disease plateau (Fig. 2), lowered the arthritis score only slightly relative to that of the adjuvant-injected controls (P not significant on day 23), with hours elapsing before any improvement was seen. Also to be noted in Fig. 2 is the gradual progression of disease to the level of the adjuvant-injected controls, which occurred after cessation of BCG administration in these groups.

(iii) Effect of long-term BCG treatment after disease induction. All control rats demonstrated the usual AD course of polyarthritis and weight loss after adjuvant injection similar to the untreated group in Fig. 2. Groups receiving BCG twice weekly, commencing, respectively, on days 6 and 12, initially showed a significant improvement in their arthritis scores. As in the preceding experiment, even with con-

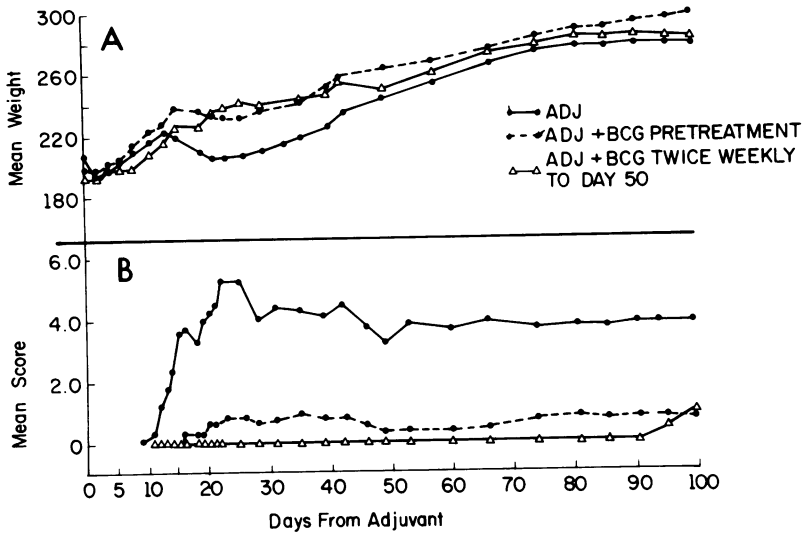

FIG. 1. *Effect of BCG on AD (10 rats per group). (A) Mean group weight (grams); (B) mean group arthritis score of the three noninjected paws.*

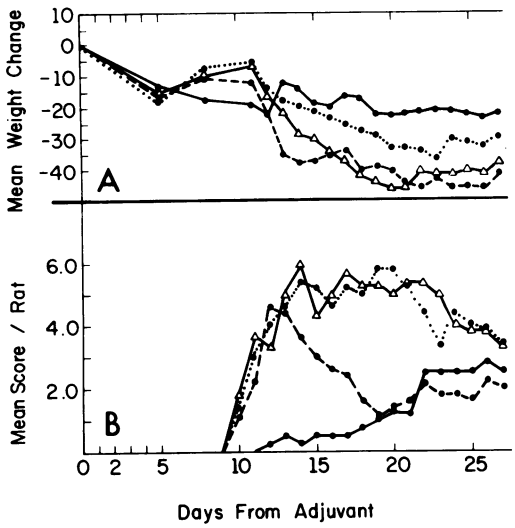

FIG. 2. *Effects of BCG given after disease induction (five rats per group). (A) Mean group weight change (grams); (B) mean group arthritis score of the three noninjected paws. BCG given on days:* (●——●) *5, 8, 11;* (●--●) *12, 15, 18;* (●···●) *19, 22, 25.* (△——△) *Control adjuvant.*

tinued twice-weekly injections of BCG, progression of arthritis was noted so that the mean score of treated animals was no different from that of untreated controls by day 40.

(iv) BCG suppression of disease induced with *C. rubrum* adjuvant. Rats given adjuvant containing *C. rubrum* developed transient erythema, swelling, and tenderness of interphalangeal and metacarpo-phalangeal joints by day 13, as reported (29). BCG started on day 11 significantly decreased the number of joints involved relative to that of the controls ($P < 0.01$ on day 20), and continued to have an effect to day 30, at which time the experiment was terminated. As in the case of the mycobacterial adjuvant, BCG pretreatment was more effective and afforded complete disease suppression between days 24 and 26 (Fig. 3).

Effect of BCG on immunological mechanisms involved in the diseases. To define cellular immune parameters which could be affected by BCG and might be of significance in explaining the observed suppression, hematological values, lymphocyte subpopulations, and PPD skin reactivity were studied.

(i) Hematological values (performed on the pretreated rats of Fig. 1). The adjuvant-injected control rats developed a chronic nonprogressive anemia which was maximal during the acute phase of the disease (Fig. 4), as has been reported (21). BCG-receiving rats developed anemia which lasted throughout treatment. Rats treated with BCG and then given adjuvant also became anemic initially, but their hemoglobin values rose toward normal more rapidly than those of the adjuvant-injected controls.

The first BCG injection produced leukocytosis, consisting mainly of mononuclear cells. After the third BCG injection a second peak of leukocytosis occurred, with mononuclear and polymorphonuclear cells contributing almost equally. A third peak appeared 3 weeks from the first BCG injection, at which point it consisted mainly of polymorphonuclear leukocytes (Fig. 5). The adjuvant-injected rats showed a post-

adjuvant leukocytosis with an increased percentage of polymorphs, followed by an absolute leukopenia with lymphocytopenia maximal during the acute phase of the disease. An increase in polymorphs was noted during the early prearthritic phase. Rats given BCG plus adjuvant also showed, after adjuvant injection, a rise in the leukocyte count related mainly to an increase in polymorphs. This group also developed a transient lymphocytopenia.

(ii) Identification and quantitation of lymphocyte subpopulations. To characterize more fully the changes in blood lymphocyte populations, the percentage of CRL (representing the major B-cell compartment in the rat) was determined (Fig. 6 and 7). The BCG-treated group showed a small rise in CRL on day 6, with normal values thereafter. The adjuvant-injected group showed an early postadjuvant increase in CRL, with a second rise during development of the polyarthritis ($P < 0.05$ on days 6 and 18 relative to normal rats). The rats receiving BCG plus adjuvant showed a marked early increase in CRL ($P < 0.01$ on day 8 relative to normal rats), with a slow return to normal.

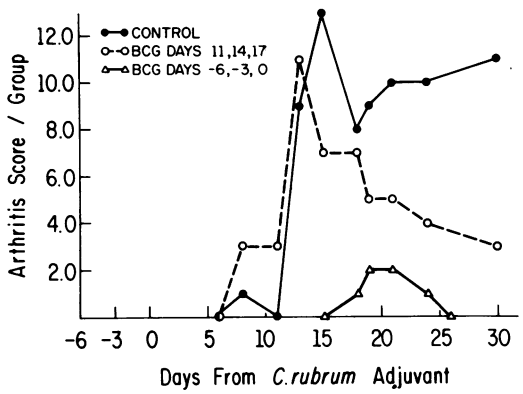

FIG. 3. *Effect of BCG on disease induced by C. rubrum adjuvant (five rats per group).*

Both the adjuvant-injected and BCG plus adjuvant-injected rats developed lymphocytopenia with concurrent increase in CRL. This lymphocytopenia was caused by a decrease in the non-CRL lymphocyte population, which in rats, as well as in humans, most likely consists largely of T-cells.

In vitro lymphocyte PHA stimulation studies are summarized in Fig. 7B and C. Figure 7B shows background [2-^{14}C]thymidine incorporation by the non-PHA-stimulated lymphocytes of the four groups. Neither adjuvant nor BCG given alone increased background incorporation (it has been found that BCG increased uptake by nonstimulated mouse spleen cultures; 43). In the rats given BCG plus adjuvant, there was increased incorporation between days 15 and 20 ($P < 0.01$ on day 20) relative to all other groups.

The PHA response shown in Fig. 7C has been plotted to compare maximal stimulation of treated groups to that of nontreated controls ($n = 20$). The mean response ± 1 standard error of this latter group, expressed as $\log_{10}$ (PHA-stimulated cells/nonstimulated cells), is 2.52 ± 0.07.

In the BCG-treated group, there was an early increase in incorporation after BCG injection. By day 6, the values fell below the control base line and remained there, showing a biphasic response which has not been reported in the mouse experiments (5). The adjuvant-injected group showed decreased response throughout the experiment. In the group receiving BCG plus adjuvant, increased thymidine incorporation was noted early, similar to the pattern of the group receiving BCG alone. The response then

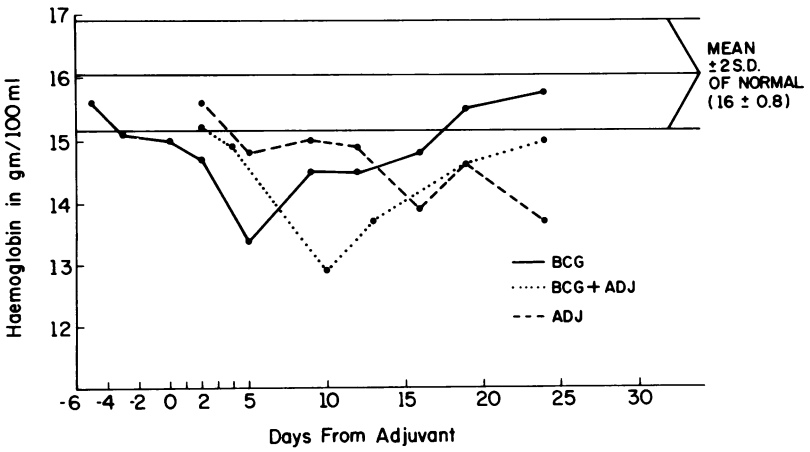

FIG. 4. *Effects of BCG on hemoglobin concentration (15 rats per group).*

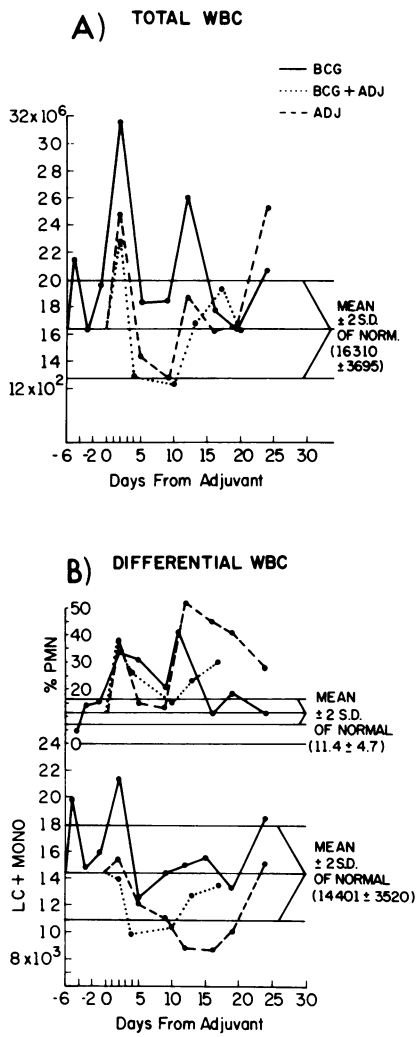

FIG. 5. *Effects of BCG on leukocyte (WBC) values (15 rats per group). (A) Total leukocyte counts; (B) differential leukocyte counts: (top) percent polymorphonuclear cells, (bottom) total mononuclear cells. Symbols as in Fig. 3.*

fell below base line by day 12 (during the most active phase), but returned to normal levels by day 18.

(iii) Lymphocyte homing experiments. Results of the TD lymphocyte homing studies are shown in Tables 1 and 2. By rosette formation with EACs it was established that 37% of TD cells collected from normal donors, and 33% of TD cells from BCG-treated donors, were CRL.

Groups 1 through 4 and 5 through 8 represent cells from normal and BCG-treated donors, respectively. Paired syngeneic recipient groups are numbered 1 and 5 (normal), 2 and 6 (BCG treated), 3 and 7 (adjuvant injected), and 4 and 8 (BCG treated plus adjuvant injected). Distribution, expressed as percentage of total counts recovered, had the same statistical validity when expressed as counts per minute per organ (Table 1) as when expressed as counts per minute per milligram of wet tissue for each of those organs (Table 2).

Group 5 when compared to group 1 showed: higher counts in thymus ($P < 0.001$), liver ($P < 0.001$), bone marrow ($P < 0.01$), and left inguinal node ($P < 0.02$); no difference in lungs ($P = 0.84$) and right inguinal nodes ($P = 0.67$); and lower counts in blood ($P < 0.002$) and spleen ($P < 0.001$).

The significant differences resulting from BCG treatment of the donors as seen in the other paired recipient groups (2 versus 6, 3 versus 7, 4 versus 8) were as follows: higher counts in the inguinal nodes of group 6 ($P < 0.001$) and lower counts in the lungs of groups 6, 7, and 8 ($P < 0.01$) relative to their respective paired groups.

Within the groups receiving cells from normal donors (groups 1 to 4) it was noted that BCG treatment of the recipients (group 2) resulted in higher thymic, lower bone marrow (both significant at the 0.001 level), and unchanged splenic counts relative to groups 1 and 3, not treated with BCG. Group 3 also had thymic counts greater than group 1, lower than group 2 ($P < 0.001$), but not different from group 4 ($P < 0.62$). Also noted in group 3 were bone marrow counts greater than in all other groups receiving normal TD cells (1, 2, 4) ($P < 0.001$), and splenic counts lower than group 1 ($P < 0.002$) but similar to groups 2 and 4.

Distribution of counts in the recipients of TD cells from BCG-treated donor rats (groups 5 to 8) was similar to that in the paired subgroups (1 to 4) receiving normal TD cells. BCG treatment of the recipients (group 6) resulted in higher thymic ($P < 0.001$) and lower bone marrow ($P < 0.001$) counts than those of normal group 5. Group 7 had thymic counts greater than those of group 5 ($P < 0.02$), but slightly lower than counts obtained in group 6 ($P = 0.1$), and bone marrow counts slightly higher than either 6 ($P = 0.09$) or 8 ($P = 0.16$). BCG treatment plus adjuvant injection of the recipients (group 8) also resulted in thymic counts greater ($P = 0.02$) and bone marrow counts lower ($P < 0.01$) than those of the normal recipients (group 5).

Additional studies in our laboratory (S. Berney, R. Bishko, and F. Quagliata, Arthritis Rheum. **14**:370, 1971; S. Berney and F. Quagliata, J. Reticuloendothel. Soc. **13**:348, 1973) suggest that the distribution of TD cells in this model varies with time, although the most

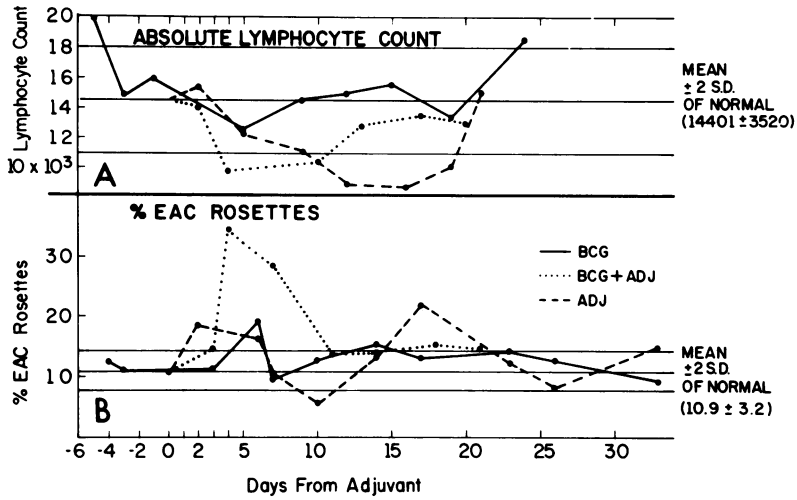

FIG. 6. *Effects of BCG on (A) total lymphocyte counts and (B) relative lymphocyte subpopulations. Five rats per treated group; 20 control rats.*

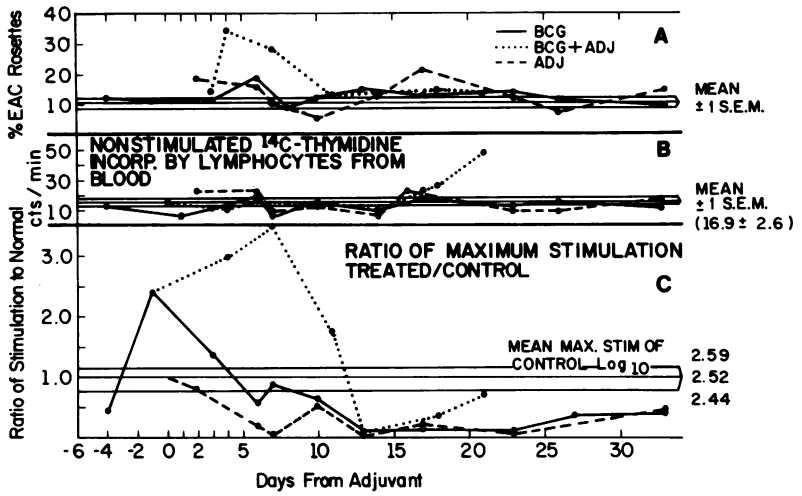

FIG. 7. *Effects of BCG on the in vitro lymphocyte response to PHA, and its temporal relation to changes in lymphocyte subpopulations. (A) Percent complement (C 3) receptors bearing lymphocytes; (B) [2-¹⁴C]thymidine incorporation by non-PHA-stimulated blood lymphocytes; (C) [2-¹⁴C]thymidine incorporation in PHA-stimulated lymphocytes relative to non-PHA-stimulated lymphocytes.*

marked differences are noted at day 5 (F. Quagliata and M. Spadaro, unpublished data).

We repeated these experiments with thymocytes from normal and BCG-treated donors; although proportionally higher counts than with TD cells were recovered in the thymi of all recipients, the general homing patterns for the various subgroups were similar.

(iv) Skin reactivity to PPD. All groups except the normal rats showed inflammation and induration around the test sites between 24 and 72 h after intradermal injection of PPD on day 14 (Table 3); no signs of inflammation or induration were present at 6 h or between 12 and 20 h, excluding the possibility of a positive Jones-Mote reaction. PPD reactivity was not determined in the experiment in which *C. rubrum* adjuvant was used.

DISCUSSION

It is known that BCG may affect the course of malignant diseases (4, 16, 26, 28). The results of the experiments reported would suggest a potential therapeutic role for BCG in nonmalignant dieseases that result from or involve immunological mechanisms. Indeed, there is already one report describing a beneficial effect of BCG in chronic rheumatoid arthritis (39), which has

TABLE 1. *Distribution of counts in syngeneic recipients of ^{51}Cr-labeled TD cells from normal and BCG-treated donor rats*

Donor rats	Recipient rats	Blood (5 ml)	Left inguinal node	Right inguinal node	Thymus	Spleen	Liver	Lungs	Femora
Untreated (normal)	Untreated (normal)	3.77 ± 0.66[a]	0.22 ± 0.12	0.38 ± 0.06	0.21 ± 0.02	58.17 ± 3.18	32.23 ± 3.24	3.59 ± 0.24	1.43 ± 0.13
	BCG pretreatment	3.12 ± 0.48	0.24 ± 0.05	0.23 ± 0.02	7.76 ± 2.04	55.53 ± 4.19	29.12 ± 1.96	3.35 ± 0.32	0.91 ± 0.17
	Adjuvant	3.24 ± 0.68	4.37 ± 0.44	0.78 ± 0.40	2.62 ± 0.88	52.35 ± 1.45	31.44 ± 0.26	3.23 ± 0.19	1.97 ± 0.24
	Adjuvant + BCG pretreatment	2.76 ± 0.12	3.23 ± 0.42	0.30 ± 0.08	8.35 ± 1.07	52.11 ± 1.97	28.90 ± 2.03	3.12 ± 0.19	1.22 ± 0.23
BCG pretreatment	Untreated (normal)	2.55 ± 0.26	0.44 ± 0.03	0.40 ± 0.08	1.98 ± 0.98	48.88 ± 0.89	40.29 ± 1.34	3.28 ± 0.31	2.18 ± 0.49
	BCG pretreatment	2.68 ± 0.23	0.40 ± 0.05	0.39 ± 0.06	8.61 ± 2.08	50.93 ± 7.77	33.60 ± 9.09	2.49 ± 0.12	1.04 ± 0.31
	Adjuvant	2.70 ± 0.34	4.99 ± 2.76	1.29 ± 0.21	5.02 ± 3.83	49.81 ± 5.79	30.51 ± 10.63	2.79 ± 0.20	2.89 ± 1.92
	Adjuvant + BCG pretreatment	2.43 ± 0.32	2.07 ± 1.98	0.35 ± 0.24	8.43 ± 4.32	53.47 ± 8.38	32.78 ± 4.54	3.61 ± 0.01	1.33 ± 0.27

[a] Values are expressed as the percentage ± standard error of total counts recovered per recipient (mean of three rats per group).

TABLE 2. *Distribution of counts in syngeneic recipients of ^{51}Cr-labeled TD cells from normal donor rats*[a]

Group	Organ	Wt (mg)	cpm	cpm/mg
Normal	L node	4.3	67.5	15.7
	R node	2.2	110.2	33.4
	Thymus	304.6	706.3	2.3
	Spleen	555.0	17,045.0	30.7
	Liver	9,716.7	9,919.0	1.0
	Lungs	1,397.0	1,050.4	0.75
BCG	L node	3.0	35.0	11.7
	R node	3.0	27.0	9.0
	Thymus	423.7	2,344.1	5.5
	Spleen	1,759.7	16,570.0	9.4
	Liver	11,396.7	8,828.3	0.77
	Lungs	1,709.7	1,006.6	0.59
Adjuvant	L node	28.3	1,396.1	49.3
	R node	6.3	253.9	40.3
	Thymus	112.0	1,164.0	10.4
	Spleen	551.0	17,043.7	30.9
	Liver	7,386.7	10,043.7	1.4
	Lungs	1,414.0	1,032.4	0.73
BCG and adjuvant	L node	30.7	713.6	23.2
	R node	—	—	—
	Thymus	300.0	2,550.7	8.5
	Spleen	1,138.7	15,859.3	13.9
	Liver	8,620.0	8,931.8	1.03
	Lungs	1,572.7	949.5	0.60

[a] Values are expressed as counts per minute per milligram of wet tissue for each organ (mean of three rats per group). —, The R nodes in this group were inadvertently discarded before they were weighed.

TABLE 3. *Rat ear skin reactivity to intradermal PPD*

Group	Reactivity[a] at:							
	6 h		24 h		48 h		72 h	
	L[b]	R[c]	L	R	L	R	L	R
Adjuvant	0	0	0	10.7	0	8.0	0	5.3
Adjuvant and BCG	0	0	0	10.6	0	10.8	0	6.0

[a] Diameter of erythema and induration (in millimeters).

[b] L, Left ear: 30 μl of diluent (Merck, Sharp & Dohme).

[c] R, Right ear: 30 μl of PPD (1,500 tuberculin units; Merck, Sharp & Dohme).

been followed by anecdotal support (20). However, the progression of disease that we observed in the AD model after cessation of treatment represents a warning against an indiscriminate use of BCG as a therapeutic agent in diseases other than malignant.

The following mechanisms could contribute to explaining the suppression of AD induced by BCG that we observed.

(i) Tolerance. Although a schedule for BCG administration capable of inducing tolerance in

the AD model has not been worked out, our experimental conditions are not compatible with those expected to induce tolerance (18, 32), as has been seen in studies involving pretreatment with *M. tuberculosis* (9, 10, 44) and/or the Wax D fraction of *M. tuberculosis* (44). It is also unlikely that tolerance-induced disease suppression (or immune paralysis of an ongoing response) would be evident as rapidly as we have observed (within 4 h) after a single injection in adult rats. Moreover, suppression of disease induced by *C. rubrum* adjuvant was also effected by BCG, and, although antibodies to some BCG antigens are to some extent cross-reactive with both *M. tuberculosis* and *C. rubrum* (45), it is improbable that this suppression occurs on the basis of tolerance. The presence of positive skin test reactivity to PPD also in the BCG-treated rats indicates that tolerance has certainly not been induced to all the antigens of *M. tuberculosis* by the BCG administration.

(ii) Antigenic competition. Without sensitive measurements of cross-reactivity between BCG and the adjuvant mixture, it is practically impossible to evaluate antigenic competition as a factor in the observed suppression. It is known that protein (9, 38), lipopolysaccharide antigens (46), and cellular (9, 10, 44, 46) as well as extracellular (38) bacterial products have suppressed AD; in most cases, these agents had to be given in the same suspension as the adjuvant mixture (9, 14). Another requirement for antigenic competition is that the competing antigens have to drain to the same regional lymph nodes (8).

We gave BCG intraperitoneally and the adjuvant mixture intradermally into the left hind paw. Thus, it is unlikely that the initial influence of BCG is on the nodes draining the injected paw. This fact, plus the reported exacerbation of AD produced by a second adjuvant injection given within 1 month of the first (10, 44), and as well as the normal humoral response in rats given protein antigen at the same time as intradermal adjuvant (38), the well-known "adjuvant" effect of Freund adjuvant even when given at a site apart from that of the antigen, suggest that antigenic competition is unlikely to be primarily responsible for the suppression reported here. Moreover, it is unlikely that the rapid improvement of arthritis (Fig. 2) in rats receiving BCG on day 12 could result from antigenic competition.

(iii) Immune complex formation. Direct measurement of anti-mycobacterial antibody was not done; however, the histological appearance of the kidney both in the *M. tuberculosis*- and in the *C. rubrum*-injected rats seemed to exclude the elimination of the antigens, as part of a complex which localizes to the kidney, as a possible mechanism of suppression (F. Quagliata and R. A. Melton, unpublished data); however, it is conceivable that immune complexes bound to tissues other than the kidney might have contributed to sequestration of the antigen.

(iv) Interferon inducer production. Reports on the suppression of AD by enhancement of interferon production and release (15) led to the hypothesis that a virus may be involved in the pathogenesis of the syndrome. BCG has indeed been shown to increase interferon production (48), and it may be capable of slowing viral replication in the AD host, with resulting suppression. However, an earlier study from our laboratory (35) tends to eliminate direct viral infection as the causative agent of AD.

(v) Cell recirculation. The significant increase in counts in the nodes and thymi in our study most likely reflects trapping of more cells than are accounted for by the usual number of antigen-specific cells in the TD population (12, 25).

It has been shown that the adult mouse thymus contains lymphocytes with specific antigen-binding receptors (25): this may explain, at least in part, both the increased trapping of TD cells in the thymus of BCG- and/or adjuvant-treated rats and the facilitated cell-cell interaction resulting from BCG treatment of donors. This might be indicative of the expansion of a T-cell population through which some of the effects of BCG that we noted might be explained.

(vi) Cell interactions. A negative influence, or suppressor function, exerted by the thymus or thymocytes stimulated by BCG should also be considered. The concurrent increases in number and percentage of CRL and increased PHA responsiveness early in the course of AD in BCG-treated rats may reflect increased helper cell activity and/or reduced influence of a suppressor T-cell subpopulation. Alternatively, the interplay between these helper cells and suppressor cells like those described in C57BL/6 mice (5, 43), in relation to the time of BCG administration and testing of the cells, might explain some of these effects. A mechanism similar to this one has been reported in explaining the enhancement of the response to type III pneumococcal polysaccharide by antithymocyte serum (2). Other immunological effects of BCG may be responsible for the observed disease suppression, particularly those affecting cellular processes involved in both afferent and efferent components of the immune response. Differentiation of mononuclear phagocytes into macrophages (1), accumulation of macrophages at the site of BCG injection (1, 6, 40), proliferation of cellular compartments of the lymphoreticular system (23, 40), and increased phagocytic activ-

ity (as measured by colloidal carbon clearance and Kupfer cell accumulation of ^{131}I-labeled human albumin) (13) have been reported in in vivo models after BCG administration.

Suppression of AD by BCG, with the observed changes in lymphocyte distribution, subpopulation ratio, and mitogen responses, may therefore also result from BCG-induced modification of thymic suppression function, thus affecting both cellular and humoral activity in the effector limb of the immune response to adjuvant. A more direct, pharmacological-type effect of the BCG should also be considered, in the light of the observed almost immediate effect on the ongoing disease. Further experiments must be done to clarify fully the suppressive mode of action of BCG.

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LITERATURE CITED

1. **Adams, D. O.** 1974. The structure of mononuclear phagocytes differentiating in vivo. I. Sequential fine and histologic studies of the effect of Bacillus Calmette-Guérin (BCG). Am. J. Pathol. **75**:17-47.
2. **Baker, P. J., R. F. Barth, P. W. Stashak, and D. F. Amsbaugh.** 1970. Enhancement of the "antibody" response to type III pneumococcal polysaccharide in mice treated with antilymphocyte serum. J. Immunol. **104**: 1313-1315.
3. **Baram, P., L. Soltysik, and W. Condoulis.** 1971. The in vitro assay of tuberculin hypersensitivity in *Macaca mulatta* sensitized with Bacille Calmette-Guérin cell wall vaccine and/or infected with virulent mycobacterium tuberculosis. Lab. Anim. Sci. **21**:727-733.
4. **Bast, R. C., Jr., B. Zbar, T. Borsos, and H. J. Rapp.** 1974. BCG and cancer (second of two parts). N. Engl. J. Med. **290**:1458-1469.
5. **Bennet, J. A., V. Srivivasa Rao, and M. S. Mitchell.** 1978. Systemic bacillus Calmette-Guérin (BCG) activates natural suppressor cells. Proc. Natl. Acad. Sci. U.S.A. **75**:5142-5144.
6. **Bhuyan, U. N., and V. Ramalingaswami.** 1973. Immune responses of the protein-deficient guinea pig to BCG vaccination. Am. J. Pathol. **72**:489-502.
7. **Bianco, C., R. Patrick, and V. Nussenzweig.** 1970. A population of lymphocytes bearing a membrane receptor for antigen-antibody complement complexes. I. Separation and characterization. J. Exp. Med. **132**:702-720.
8. **Brody, N. I., and G. W. Siskind.** 1972. Studies on antigenic competition. II. Evidence for effect at level of antigen processing. Immunology **22**:75-85.
9. **Flax, M. H., and B. H. Waksman.** 1963. Further immunologic studies of adjuvant disease in the rat. Int. Arch. Allergy Appl. Immunol. **23**:331-347.
10. **Gery, I., and B. H. Waksman.** 1967. Studies of the mechanism whereby adjuvant disease is suppressed in rats pretreated with mycobacteria. Int. Arch. Allergy Appl. Immunol. **31**:57-68.
11. **Glenn, E. M.** 1966. Adjuvant-induced arthritis: effects of certain drugs on incidence, clinical severity, and biochemical changes. Am. J. Vet. Res. **27**:339-352.
12. **Gowans, J. L., and E. J. Knight.** 1964. The route of recirculation of lymphocytes in the rat. Proc. R. Soc. London Ser. B **159**:257-282.
13. **Howard, J. G., G. Biozzi, B. N. Halpern, C. Stiffel, and D. Mouton.** 1959. The effect of *Mycobacterium tuberculosis* (BCG) infection on the resistance of mice to bacterial endotoxin and *Salmonella enteritidis* infection. Br. J. Exp. Pathol. **40**:281-290.
14. **Isakovic, K., and B. H. Waksman.** 1965. Effect of sensitization to BSA on adjuvant disease in normal and neonatally thymectomized rats. Proc. Soc. Exp. Biol. Med. **119**:676-678.
15. **Kapusta, M. A., and J. Mendelson.** 1969. The inhibition of adjuvant disease in rats by the interferon-inducing agent pyran copolymer. Arthritis Rheum. **12**:463-471.
16. **Klein, E., and B. G. Leventhal.** 1973. Human studies, p. 139. *In* T. Borsos and H. J. Rapp (ed.), National Cancer Institute Monogr. 39. U.S. Department of Health, Education and Welfare, Bethesda, Md.
17. **Lance, E. M., P. J. Ford, and M. Ruszkiewicz.** 1968. The use of subcellular fractions to raise anti-lymphocytic serum. Immunology **15**:571-580.
18. **Leech, S. H., and N. A. Mitchison.** 1976. Break-down of tolerance. Br. Med. Bull. **32**:130-134.
19. **Lennon, V. A., and W. S. Byrd.** 1973. Experimental arthritis in thymectomized rats with an impaired humoral response. Nature (London) **244**:38-40.
20. **LeVay, D.** 1974. Tuberculin in rheumatoid arthritis. Lancet **ii**:908.
21. **Lukens, J. N., G. E. Cartwright, and M. M. Wintrobe.** 1967. Anemia of adjuvant-induced inflammation in rats. Proc. Soc. Exp. Biol. Med. **126**:346-349.
22. **Mackaness, G. B., C. J. Auclair, and P. H. Lagrange.** 1973. Immunopotentiation with BCG. I. Immune response to different strains and preparations. J. Natl. Cancer Inst. **51**:1655-1667.
23. **Miller, T. E., G. B. Mackaness, and P. H. Lagrange.** 1973. Immunopotentiation with BCG. II. Modulation of the response to sheep red blood cells. J. Natl. Cancer Inst. **51**:1669-1676.
24. **Mitchell, M. S., D. Kirkpatrick, M. B. Mokyr, and I. Gery.** 1973. On the mode of action of BCG. Nature (London) New Biol. **243**:216-217.
25. **Modabber, F., S. Morikawa, and A. H. Coons.** 1970. Antigen-binding cells in normal mouse thymus. Science **170**:1102-1104.
26. **Morton, D. L., F. F. Eilber, R. A. Malmgren, and W. C. Wood.** 1970. Immunological factors which influence response to immunotherapy in malignant melanoma. Surgery **68**:158-164.
27. **Okuyama, H., and K. Morikawa.** 1972. Plaque-forming cells in rabbits immunized with BCG bacilli. Infect. Immun. **6**:893-898.
28. **Old, L. J., D. A. Clarke, and B. Benacerraf.** 1959. Effect of bacillus Calmette-Guérin infection on transplanted tumors in the mouse. Nature (London) **184**: 291-292.
29. **Paronetto, R.** 1970. Adjuvant arthritis induced by *Corynebacterium rubrum*. Proc. Soc. Exp. Biol. Med. **133**: 296-298.
30. **Pearson, C. M., and F. D. Wood.** 1959. Studies of polyarthritis and other lesions induced in rats by injection of mycobacterial adjuvant. I. General clinical and pathologic characteristics and some modifying factors. Arthritis Rheum. **2**:440-459.
31. **Pearson, C. M., and F. D. Wood.** 1964. Inhibition of adjuvant arthritis by some protein antigens. Arthritis Rheum. **7**:337.
32. **Phillips-Quagliata, J. M.** 1972. Cellular events in tolerance. I. Induction and loss of tolerance in neonatal and adult rats. Cell. Immunol. **4**:116-133.
33. **Quagliata, F., D. Faig, M. Conklyn, and R. Silber.** 1974. Studies on the lymphocyte 5'-nucleotidase in chronic lymphocytic leukemia, infectious mononucleosis, normal subpopulations, and phytohemagglutinin-stimulated cells. Cancer Res. **34**:3197-3202.

34. **Quagliata, F., V. J. W. Lawrence, and J. M. Phillips-Quagliata.** 1973. Prostaglandin E_1 as a regulator of lymphocyte function: selective action on B lymphocytes and synergy with procarbazine in depression of immune responses. Cell. Immunol. **6**:457–465.
35. **Quagliata, F., and J. M. Phillips-Quagliata.** 1972. Competence of thoracic duct cells in the transfer of adjuvant disease and delayed hypersensitivity. Evidence that mycobacterial components are required for the successful transfer of the disease. Cell. Immunol. **3**: 78–87.
36. **Quagliata, F., J. M. Phillips-Quagliata, and G. L. Floersheim.** 1972. Immunosuppression by procarbazine. I. Sites of action of the drug and effect on adjuvant arthritis and circulating antibody response. Cell. Immunol. **3**:198–212.
37. **Quagliata, F., P. M. Sanders, and D. L. Gardner.** 1969. Suppression of adjuvant arthritis by a new cytotoxic compound, Rubidomycin. Ann. Rheum. Dis. **28**:163–171.
38. **Quagliata, F., and A. Taranta.** 1972. Suppression of adjuvant disease by bacterial extracellular products. Ann. Rheum. Dis. **31**:500–507.
39. **Rewald, E.** 1974. BCG in rheumatoid arthritis. Lancet **ii**: 785–786.
40. **Rosenthal, S. R.** 1973. *In* T. Borsos and H. J. Rapp (ed.), National Cancer Institute Monogr. 39, p. 91–104. U.S. Department of Health, Education and Welfare, Bethesda, Md.
41. **Sorber, W. A., E. S. Leake, and Q. N. Myrvik.** 1973. Comparative densities of hydrolase-containing granules from normal and BCG-induced alveolar macrophages. Infect. Immun. **7**:86–92.
42. **Taranta, A., G. Cuppari, and F. Quagliata.** 1969. Dissociation of hemolytic and lymphocytic transforming activities of streptolysin S preparations. J. Exp. Med. **129**:605–622.
43. **Turcotte, R., L. Lafleur, and M. Labreche.** 1978. Opposite effects of BCG on spleen and lymphnode cells: lymphocyte proliferation and immunoglobulin synthesis. Infect. Immun. **21**:696–704.
44. **Waksman, B. H., C. M. Pearson, and J. T. Sharp.** 1960. Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant. II. Evidence that the disease is a disseminated immunologic response to exogenous antigen. J. Immunol. **85**:403–417.
45. **Wolinsky, E.** 1969. Mycobacteria, p. 843–845. *In* B. D. Davis, R. Dulbecco, H. N. Eisen, H. S. Ginsberg, W. B. Wood, Jr., and M. McCarty (ed.), Microbiology, 2nd ed. Harper & Row, New York.
46. **Wood, F. D., and C. M. Pearson.** 1962. Protection of rats against adjuvant arthritis by bacterial lipo-polysaccharides. Science **137**:544–545.
47. **Yamamoto, K., and Y. Takahashi.** 1971. Macrophage migration inhibition by serum from desensitized animals previously sensitized with tubercle bacilli. Nature (London) New Biol. **233**:261–263.
48. **Youngner, J. S., and S. B. Salvin.** 1973. Production and properties of migration inhibitory factor and interferon in the circulation of mice with delayed hypersensitivity. J. Immunol. **111**:1914–1922.
49. **Zurier, R. B., and F. Quagliata.** 1971. Suppression of adjuvant arthritis by prostaglandin E_1. Nature (London) **234**:304–305.